charge of the interface. Recently, charge inversion has been reported in the bacterial channel OmpF, in the presence of salts of divalent cations [Alcaraz et al. Biophys. J. 96 (2009) 56]. Aiming to get an insight on the atomistic mechanism of the cation interaction with the protein, we have performed extensive MD simulations of a realistic model of the OmpF WT protein in a POPC membrane in MgCl2 and explicit water. The simulations were computationally highly demanding, with half million atoms in a simulation box and production runs around 25 nsec.
The simulations were performed employing the NAMD simulation package running in 128 processor at the CESGA Supercomputing Center. Our main result is that we have observed charge inversion of certain important acidic groups. The observed charge inversion is accompanied by a change in the transport mechanism of ions inside the channel and a reversal in the selectivity of the channel. Overall, our simulations give an accurate microscopic image of this unexpected effect with potentially important biological and nanotechnological implications.

1731-Pos
Increased Salt Concentration Promotes Negative Cooperativity in OmpF Channel
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The concept of positive cooperativity appeared in the study of oxygen uptake by hemoglobin to explain that when a molecule of oxygen binds makes it easier for a second molecule to bind. Quite the reverse, negative cooperativity refers to the situation where the presence of the first molecule makes it more difficult for the second molecule to bind. We study here the effect of salt on the pH titration of the OmpF channel, paying attention to the current noise, conductance and ion selectivity that are analyzed in terms of the Hill formalism. In all cases, values lower than 1 are found, suggesting a negative cooperativity. Although OmpF porin is a trimer, it was shown by a number of different methods that each monomer is identical and functionally independent. Thus, the slowed-down channel titration is a property of each monomer. Surprisingly, we find that increasing salt concentration promotes negative cooperativity, which is seen as a salt-induced decrease of the Hill coefficient. This observation seems to exclude direct electrostatic interactions between protonation sites as the source of the phenomenon, suggesting another, more subtle mechanism(s). The binding of cations to certain acidic residues has a crucial effect at low pH because results in an inhibition of channel conductance that additionally provides an anionic selectivity to the channel. This suggests that the binding site could play a certain role in the protection of the bacteria against acidic media

1732-Pos
Anions from the Hofmeister Series: Single Molecule Detection with a Solitary Protein Nanopore
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Nanopores have emerged in recent years as versatile single molecule detectors. The sensing principle is based on transient interruptions in the ion-current of an electrolyte, induced by the entry, transport, and exit of a particular analyte from the pore. The improving the detection capability of the nanopore is essential. Recently (Rodrigues et al., 2008) we have shown that the ''salting out'' are responsible for the KCl-induced enhancement in identification of individual molecules of poly(ethylene glycol) using solitary α-hemolysin nanoscale pores. The result suggested that specific ion effects may take place. Hofmeister effects are almost ubiquitous (Lo Nostro et al., 2006). Despite the huge number of studies devoted to this issue that date back more than a century, their origin is still debated. There are only isolated studies of the phenomenon at the confined spaces. For this reason, we focused on the effect of monovalent anions on a simple bimolecular complexation reaction between poly(ethylene glycol) and α-hemolysin nanoscale pore at the single-molecule level.
We find that the type of anions used here has dramatic influence on the ''on-rate'' constant of the reaction (the difference reaches several hundred times). As a consequence of this, the transition rate and the detection limit of the nanopore based sensor is correspondingly changed. The all probed anions follow the Hofmeister ranking according to their influence on the on-rate constant ($F^- > Cl^- > Br^- > I^-$) and the solubility of the analyte ($F^- < Cl^- < Br^- < I^-$). Therefore, salting-out phenomenon is responsible for the anion-induced effect on single molecule detection with a solitary protein nanopore. These results will advance the development of devices with sensor elements based on single nanopores.
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1733-Pos
Extension of Poisson-Nernst Planck Theory of Ion Conductivity with Soft-Repulsion Potential between Ions and Protein. Sensitivity of I-V Properties of α-Hemolysin Channel on its Penetration Depth into Membrane
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A soft repulsion (SR) potential between mobile ions and protein atoms is introduced to Poisson-Nernst-Plank (PNP) theory of ion transport as an alternative to commonly used hard sphere repulsion (HR). Two sets of SR were tested: one is parameterized for all atoms of 20 essential amino-acid residues using full atomic molecular dynamic simulation (SR-MD); and another is a truncated Lennard-Jones potential (SR-LJ). The effect of different models of short-range interaction between protein atoms and mobile ions (HR, SR-MD and SR-LJ) were studied using α-hemolysin channel protein. In addition, four different methods of setting the diffusion coefficients were analyzed in order to evaluate the effect of diffusion distribution on predicted currents. Our calculations show that the diffusion distribution has a strong influence on the size of total currents whereas has significantly less effect on rectifications, reverse potentials and selectivity. Therefore, for proper modeling of these properties, the potential of mean force (PMF) may play a more important role than the diffusion distribution. SR-MD has a better approximation of PMF near the protein surface than HR and significantly improves selectivity predictions.
Additionally, we have studied the dependency of α-hemolysin I-V properties on the penetration depth of the channel into the membrane. The results show that rectification and reverse potentials are very sensitive to the penetration depth. The depth, predicted by matching calculated rectification with the experimentally determined one, is in a very good agreement with the neutron reflection experimental result. Our free energy estimation also indicates that there is a minima near the predicted depth.

1734-Pos
Monitoring Ion Channel Charge Displacements using Radio Frequencies
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Here we introduce a new technique to examine voltage-dependent ion-channel biophysics using radio frequency (RF) interrogating electric fields. The approach exposes the cell membrane to an RF electric field and measures vibrational electric current evoked by the RF field. *Xenopus* Oocytes transfected to express Shaker-B IR ion channels were used as the experimental model. A 500 kHz RF signal was applied to the membrane using extracellular bipolar metal electrodes, and RF charge displacement measurements were made during traditional two-electrode whole-cell voltage clamp. Voltage clamp was used to depolarize the oocytes and measure whole-cell K^+ current at several transmembrane potentials. The RF interrogation signal was superimposed on top of the comparatively slow (DC) voltage clamp command signal. Results show that the measured RF membrane current was a function of DC membrane potential. The RF current was separated into conduction and displacement components to examine the voltage-dependent RF conductance, G_{RF}, and capacitance, C_{RF}. Remarkably, the RF capacitance, C_{RF}, had a voltage sensitivity and half-activation voltage that correlated with the Shaker-B IR channel DC conductance measured using whole-cell voltage clamp. These data are consistent with the hypothesis that electrostatic interactions between the channel protein and K+ in the pore constrain the mobility of K+ and lead to changes in RF capacitance with membrane depolarization. The approach might offer a means to examine electrostatic interactions associated with ion channel function or to estimate voltage dependence of channel activation using extracellular RF signals. [supported by NIH R01DC04928, NSF IGERT DGE-9987616]